Cilia: Movers, Shakers, and Receivers

15Jan2016

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Outline

1. What are cilia?

2. Ciliogenesis

3. Ciliopathies

4. Trafficking in and out of cilia

5. Primary cilia as antennas
We have known about flagella for hundreds of years...
Kartagener’s Syndrome:
defective dynein arms = bronchial infections, immobile sperm, situs inversus

But it wasn’t until 1976 that a physical defect was attributed to immobile sperm

Yes. Eukaryotic motile cilia are pretty similar to eukaryotic flagella.

Cilia are generally shorter (~6-10 μm) than flagella (~150 μm)

The term “Intraflagellar transport” (IFT) applies to both flagella and cilia.
The motions of cilia and flagella are different, although the gross features are similar.

Cells that are multi-ciliated tend to have their cilia beat in a coordinated fashion.
A brief digression on prokaryotic flagella

Prokaryotic flagella are structurally different from eukaryotic flagella/cilia.

Motion is driven by a rotary motor mechanism, like a crank.

The filament component of bacterial flagella is a protein called flagellin.

Recall that flagellin is recognized by the innate immune system in both plants and animals.

**Prokaryotic Flagellum**

No membrane

The rotor is driven by a proton pump

Rotary Engine

Assembly by a secretion type system

Archaeal Flagella resemble bacterial flagella, but the composition and assembly are different.

The surprisingly diverse ways that prokaryotes move
Ken F. Jarrell & Mark J. McBride
Nature Reviews Microbiology 6, 466-476 (June 2008)
What types of organisms have cilia/flagella?

- Animals? ✔
- Fungi? ✔ (some species, e.g., chytrids, have motile gametes)
- Bacteria? ✔ (these are entirely different from eukaryotic flagella)
- Unicellular Algae (Chlamydomonas)? ✔
- Protozoans (Tetrahymena)? ✔
- Archaebacteria? ✔ (these are entirely different from eukaryotic flagella)
- Plants? ✔ (Some species, like Ginko and moss, have motile sperm)
1896, Sakugoru Hirase described "Spermatozoid of Ginkgo biloba": motile sperm

Prof. Jin Murata

“The symbolic Ginkgo tree from which zoospore was discovered”

https://s10.lite.msu.edu/res/msu/botonl/b_online/fo47/ginkgo/ogura.htm
Model Organisms for structural studies of cilia and flagella

Tetrahymena
(a Ciliate)

Chlamydomonas

Other model organisms include:
Trypanosomes, Drosophila, C. elegans, zebrafish, mice, dog (MDCK cells), and cultured human cells (e.g., retinal pigmented epithelial cells). Even HEK293 cells have primary cilia, but they are a bit small and hard to visualize.

Sea Urchin sperm

George Watchmaker
Lawrence Livermore National Laboratory
Diversity of cilia types in vertebrates (e.g., mammals).


Ca. 1961

mid-1950s

Ca. 1956

1679

(Association with clearance of the airway:1930s)

Why was there an explosion of articles on cilia in the 1950s and 1960s?

Choksi S P et al. Development 2014;141:1427-1441

dev.biologists.org
Animals: where can we find cilia?

Not all 9+2 cilia are motile, and not all 9+0 cilia are non-motile

Almost all mammalian cells are ciliated—with either specialized cilia or primary cilia.


He et al. (2014) Mol Biol Cell. 25:1715-29
Motile cilia are generally “9+2” and primary cilia are generally “9+0”
Review of Microtubules, Dynein, and Kinesin

1. **Microtubules**: heterodimers of α-tubulin and β-tubulin, both of which bind GTP. Microtubules are a major part of the intracellular cytoskeleton, and are involved in localization of organelles, intracellular transport, as well as the mitotic spindle.

   - **Polarity**: there is a – end and a + end. Hydrolysis of GTP to GDP destabilizes microtubules.

   - The minus end (α) is (often) anchored in the basal body, and the plus end (β) is the growing end.

   - In the cytoskeleton, microtubules are built from α-β heterodimers that form a hollow tube with 13 units per helical repeat.

   - In axonemes, the peripheral microtubules have a doublet structure, 13 in ring A plus 10 in ring B.
The microtubules in axonemes grow at the “+” tip
As early as the 1980s, people speculated that different isoforms would confer different properties to microtubules.

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<tr>
<th>α-Tubulin</th>
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<td>TUBB*</td>
<td>TUBG1*</td>
<td>TUBD1</td>
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<td>TUBA1B</td>
<td>TUBB1(*)</td>
<td>TUBG2</td>
<td>ε-tubulin</td>
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<td>TUBB2B*</td>
<td>TUBGCP3</td>
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<td>TUBB2C</td>
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<td></td>
<td>TUBB6</td>
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</tbody>
</table>

* Mutations associated with human genetic diseases

- With post-translational modifications and differentially expressed isoforms, we have a large amount of combinatorial diversity.

Keep in mind that much of what we know is based on model organisms.

In Flies, all 9+2 motile axonemes utilize α84B α-tubulin plus β2 β-tubulin.

In worms, sensory cilia work optimally with TBA-6 and TBA-9 α-tubulins, and TBB-4 β-tubulin.
The C-termini of α-tubulin and β-tubulin are post-translationally modified.

- HDAC6 deacetylates α-tubulin during cilia disassembly.
- In cycling cells, cilia are disassembled, and the centrioles organize the mitotic spindle during M phase.

Overexpression of a Dominant-negative Glycyl-ligase, TTLL3 causes defective axoneme assembly in Tetrahymena

B-G = Dominant Negative overexpressing axonemes

Wloga D et al. (2009) Dev Cell 16:867-76
Depletion of TTLL3 Produces Defects in the LR Asymmetry in Zebrafish

A tubulin monoglycylase is required for proper L-R symmetry breaking

Lefty is a TGFβ family member that determines LR Asymmetry

cmlc2 (cardiac myosin light chain 2) is a marker of heart development

Wloga D et al. (2009) Dev Cell 16:867-76
2. Dyneins

Cilia have 2 types of dyneins, Cytoplasmic and Axonemal

- **Cytoplasmic Dynein**: this is a large (~1.5 Mda) multisubunit motor protein that “walks” along microtubules, and carries out Retrograde Intraflagellar Transport (IFT) of various cargos

https://www.youtube.com/watch?v=-7AQVbrmzFw
• Ciliary (Axonemal) Dynein is the motor that bends cilia
3. Kinesins

- Anterograde IFT

Multisubunit motor proteins that “walk” along microtubules toward the + end (anterograde) and deliver cargos (e.g. tubulin, dynein, receptors) to the cilia tip.

https://www.youtube.com/watch?v=y-uuk4Pr2i8
Ciliogenesis commonly occurs as cells exit the cell cycle (G₀).

(in the lab, starving cells by limiting serum promotes ciliogenesis)

Cilia grow out of the basal body, derived from the mother centriole. Positioning, orientation, and docking of the basal body are tightly regulated.

Axonemes are initiated by adding tubulin subunits to the distal end of the basal body and then extending by adding tubulin at the tip. That means that IFT is essential for ciliogenesis.

Two types of transcription factors, the RFX family and FoxJ1, are involved in triggering ciliogenesis.
Direct and indirect targets of ciliary transcription factors.

Choksi S P et al. Development 2014;141:1427-1441
Multiple pathways of ciliogenesis.

RFX target genes are required for motile and non-motile cilia

RFX target genes are required for docking of the basal body to the apical membrane

FoxJ1 target genes are required for motile cilia

Forming multiple cilia involves duplicating centrioles

Structural studies and microscopy have localized many **centrosomal** proteins

Some of these, like CEP164, are essential for docking the mother centriole to the membrane.

PLK4, a kinase involved in centriole duplication is also required for ciliogenesis. One target is TUBGCP6.
Steps of Ciliogenesis

1. PCV = Primary Ciliary Vessicle
2. V = 2° vesicles fuse with the PCV
3. CiPo = Ciliary Pocket; invagination of the PCV
4. PM = Plasma Membrane

DA = Distal Appendages (Includes CEP164)

CEP164 is required for docking of the PCV

CB = Ciliary Bud. The cap protein CP110 is released by TTBK2

The membrane composition of the ciliary membrane is different from the plasma membrane.

What determines the length of cilia?

IFT probably has a lot to do with it (rate of anterograde assembly versus rate of retrograde disassembly), but there are sure to be other factors...

Size of pools of precursors

A large siRNA-based screen in human retinal pigmented epithelial cells identified expected genes, like KIF3A (no cilia!), a kinesin

Is there a connection to actin??

Surprisingly, depletion of 2 gelsolin-family proteins reduced cilia numbers.

gelsolin regulates actin organization by cleaving actin filaments

Depletion of an ARP3 homolog, which is required for actin branching, lengthened cilia, suggesting that branched actin networks inhibit ciliogenesis

**NCBI Annotation: Confirmed negative ciliogenesis modulators (13 genes)**

<table>
<thead>
<tr>
<th>Entrez GeneID</th>
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<th>On Cilium</th>
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Depletion of PLA2G3 a phospholipase, lengthened cilia, suggesting the involvement of recycling endocytosis in cilia length determination

Overexpressed PLA2G3 inhibited ciliogenesis.

(phospholipases have been proposed to induce curvature of membranes, and may be indirectly involved in endosome trafficking)

Ciliopathies
Human Genetics and ciliopathies

1. Primary Ciliary Dyskinesia (includes Kartagener’s Syndrome)

2. Bardet-Biedl Syndrome

3. Joubert Syndrome

Others: Nephronophthisis (Senior-Loken syndrome); Polycystic Kidney disease; Retinitis pigmentosa; Alstrom Syndrome; OCRL, etc.
Kartagener’s Syndrome:
defective dynein arms = bronchial infections, immobile sperm, situs inversus

Primary Ciliary Dyskinesia

1. Frequent lung infections
2. Male infertility
3. When accompanied by Situs inversus = Kartegener’s
4. Other, variable features: poor sense of smell; poor hearing

Primary Ciliary Dyskinesia affects cilia in the airway, making it hard to clear junk out of the lungs, leading to frequent lung infections.
Motile cilia are generally “9+2” and primary cilia are generally “9+0”.
Mutations* in subunits of dynein, or components of the axoneme, or assembly factors can lead to immobile cilia.

TABLE 1. GENES THAT CAUSE PRIMARY CILIARY DYSKINESIA

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Axonemal Component</th>
<th>Ciliary Ultrastructure of Patients with PCD with Biallelic Mutations</th>
<th>OMIM*</th>
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<td>DNAHS</td>
<td>ODA-HC</td>
<td>ODA defects</td>
<td>603335</td>
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<td>ODA IC</td>
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<td>DNAI2</td>
<td>ODA IC</td>
<td>ODA defects</td>
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<td>TXNDC3</td>
<td>ODA LC/IC</td>
<td>Partial ODA defects†</td>
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<td>K10</td>
<td>Cytoplasmic$^+$</td>
<td>ODA+IDC defects</td>
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<td>RSPH4A</td>
<td>RSH</td>
<td>Tx and CP defects</td>
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<td>RSPH9</td>
<td>RSH</td>
<td>CP defects or normal</td>
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<td>CCDC39</td>
<td>DRC</td>
<td>Microtubule disorganization$^+$</td>
<td>Current*</td>
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<tr>
<td>CCDC40</td>
<td>DRC</td>
<td>Microtubule disorganization$^+$</td>
<td>Current*</td>
</tr>
</tbody>
</table>

Definition of abbreviations: CP = central pair; HC = heavy chain; IC = intermediate chain; IDA = inner dynein arm; LC = light chain; ODA = outer dynein arm; PCD = primary ciliary dyskinesia; RS = radial spoke head; Tx = transposition.

DRC = Dynein Regulatory Center

† Partial defect with approximately two thirds of cilia with shortened or absent and one third with normal ODA.
$^+$ Cytoplasmic proteins required for the dynein arms assembly.
$^+$ Microtubule disorganization characterized by reduced number of IDA, eccentric CP, abnormal alignment of outer doublets, and occasional displacement of outer doublet.

*Interestingly, this is an autosomal recessive disorder

Situs Inversus—What is it, and what’s the connection to cilia?
How is L-R asymmetry established?

(Right lateral plate mesoderm) (Left lateral plate mesoderm)

Diego Franco, Jorge N Domínguez, Amelia Aránega.
Left-Right Asymmetry: clockwise twirling of cilia creates a Right-to-Left flow across the Node

Macromolecules $>\sim 15$ kDa are moved along the current

Okada et al., Cell 121, pp.633-644
Left-right asymmetry determined by currents generated by cilia

Posteriorly Tilted Rotation of Cilia of the Mouse Nodal Pit

Okada et al., Cell 121, pp.633-644
Nodal cilia establish an asymmetric distribution of a fluorescent macromolecule

Planar Cell Polarity mechanisms establish the orientation and angle of the cilia

Okada et al., Cell 121, pp.633-644
Bardet-Biedl Syndrome

Phenotype: postaxial polydactyly, mental retardation, hypogonadism, renal dysfunction, retinal degeneration, situs inversus, obesity

How are these features connected?

Letters to Nature

Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome

Stephen J. Ansley1,8, Jose L. Badano1,8, Oliver E. Blacque3,8, Josephine Hill1, Bethan E. Hoskins1,2, Carmen C. Leitch1, Jun Chul Kim3, Allison J. Ross3, Erica R. Eichers5, Tanya M. Teslovich1, Allan K. Mah3, Robert C. Johnsen3, John C. Cavender7, Richard Alan Lewis5,6, Michel R. Leroux3, Philip L. Beales4, Nicholas Katsanis1,2

This was a key publication that heralded a wave of publications collectively describing “ciliopathies”
The BBSome

- Discovered by Localization and tandem affinity purification

7 BBS proteins co-purify

BBS5 binds phosphatidyl inositol triphosphate

Genotype-Phenotype correlations—BBS8

Different mutations in the same gene can cause different phenotypes

Bardet-Biedl Syndrome:
- obesity, retinitis pigmentosa, polydactyly, hypogonadism, renal disease

15 different genes, and counting, plus some modifier genes

Some clinical features overlap with other diseases, e.g., Meckel Syndrome, Alstrom Syndrome
The BBSome is an integral element of anterograde IFT

How does “Turnaround” at the tip of the cilia happen?

Defects in the BBSome could affect localization of receptors. How would this explain some of the phenotypes of BBS?
A point mutation in BBS1 *partially* impairs binding of the BBsome to IFT-A/B, slowing the return of IFT components to the cytoplasm by retrograde transport.

A point mutation in DYF-2 *partially* impairs binding of the BBsome to IFT-A/B, slowing the return of IFT components to the cytoplasm by retrograde transport.

All drawings of IFT are oversimplified!

Much of Cilia diversity is governed by IFT. What governs the selectivity of kinesins for cargo?

Is there a Ciliary Targeting Signal (CTS)?

How do you keep unwanted cytoplasmic proteins from entering the cilia?
Cilia are primary Antennae for many cells

Many receptors, such as GPCRs, are preferentially localized to cilia.

- Olfactory receptors

- (Some) Neuronal receptors (somatostatin receptor 3; serotonin receptor 6; dopamine receptors, etc.)

Omori et al. looked for ciliary localization of 138 GPCRs in brain, but only found 12/138 that were reproducibly ciliary. Kind of interesting, although a bit disappointing...

Cilia organize several signaling receptors, including Patched

...also the leptin receptor
Joubert Syndrome/Cerebrooculorenal Syndrome

Hypoplasia of cerebellar vermis ("molar tooth sign"), retinal dysplasia (blindness), renal disease. This affects balance and coordination (hypotonia), development, and cognitive function. Features can be variable in severity. Affected genes include \textit{INPP5E}, \textit{TMEM216}...

Autosomal recessive, genetically heterogeneous, with 20 loci identified (JBTS1-20). A recently described mutation in CEPI41 affects localization of a tubulin glutamylase (TTLL6) to cilia. (Centrosomal Protein 41)

\[http://globalgenes.org\]
What’s the connection with mental retardation?

Cilia are antennae for sensing the environment

The primary cilia are packed with receptors (GPCRs, etc.)

Defects in cilia disrupt signaling during neural development

- cerebellum
- hippocampus
- axonal guidance

Motile cilia circulate cerebrospinal fluid—recall that ependymal cells line ventricles
Another example of signaling in primary cilia


A role for primary cilia in glutamatergic synaptic integration of adult-born neurons.

Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York, USA.

Abstract
The sequential synaptic integration of adult-born neurons has been widely examined in rodents, but the mechanisms regulating the integration remain largely unknown. The primary cilium, a microtubule-based signaling center, is essential for vertebrate development, including the development of the CNS. We examined the assembly and function of the primary cilium in the synaptic integration of adult-born mouse hippocampal neurons. Primary cilia were absent in young adult-born neurons, but assembled precisely at the stage when newborn neurons approach their final destination, further extend dendrites and form synapses with entorhinal cortical projections. Conditional deletion of cilia from adult-born neurons induced severe defects in dendritic refinement and synapse formation. Deletion of primary cilia led to enhanced Wnt and β-catenin signaling, which may account for these developmental defects. Taken together, our findings identify the assembly of primary cilia as a critical regulatory event in the dendritic refinement and synaptic integration of adult-born neurons.
Exosomes are a hot topic right now

Do cilia release exosomes from the tips?
Model illustrating unidirectional trafficking of SAG1 during ciliary adhesion and signaling. SAG1* in resting cells is present at the cell periphery, with only small amounts in cilia.

Chlamydomonas mating may involve release of exosomes from the tips of the flagella.

Other groups have proposed models where GPCRs in mammalian cells are released from cilia by exosomes, but this idea needs more supporting evidence (one paper was retracted!)

Muqing Cao et al. eLife Sciences 2015;4:e05242
Cilia and Cancer

Many types of cancer show a loss of cilia:

- Prostate Cancer
- Renal Cell Carcinoma
- Pancreatic Cancer
- Ovarian Cancer
- Melanoma
- Breast Cancer

There are at least 2 explanations:

1. Rapidly proliferating cancer cells never exit the cell cycle

2. A hallmark of cancer is genomic instability and aneuploidy, which is a hallmark of aberrant mitoses; this could result from defective centrosomes. Defective centrosomes may be impaired for ciliogenesis.
In addition to ORC proteins associated with centrosomes, there are several reports of DNA repair proteins associated with centrosomes:

- Gen1 (resolvase)
- FANCI (ICL repair)
- BRCA2 (HR)
- BRCA1 (HR)
- TopBP1 (DSB repair)
- NBS1 (end resection)
- Rad51 (HR)
- Rad51 paralogs (HR)
- PARP1 (DDR)
- ATM (DDR checkpoint)
- ATR (DDR and replication checkpoint)
- Chk2 (DDR checkpoint)
Main Points

• Cilia are highly complex organelles (300-600 proteins)

• Cilia can be both motile or non-motile

• Motile cilia are required for cell movement, establishing flow over a surface, or clearance of particles from the airway

• The ciliary membrane is distinct from the plasma membrane. This allows the assembly of a distinct cell signaling environment, with specific receptors localized to the ciliary membrane

• Many human genetic diseases, “ciliopathies”, are caused by defects in either ciliogenesis or ciliary function